The
Glen Report
Newsletter

GLEN RESEARCH
part of Maravai LifeSciences

Volume 32.1 | May 2020

Thiazole Orange as a Fluorogenic Reporter in Oligonucleotide Probes

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Fluorescence is used extensively in nucleic acid-based applications such as DNA sequencing, real-time PCR and genome imaging.[1,2] Well-established fluorescence systems for nucleic acid sequence recognition include TaqMan probes,[3] Molecular Beacons,[4] Scorpion primers[5] and HyBeacons.[6] In all these cases, a fluorophore is combined with a quencher (fluorescent or non-fluorescent) to allow fluorescence enhancement in the presence of the target sequence via separation of the fluorophore from the quencher. Improvements in fluorogenic hybridisation probe methodologies have great potential in the field of nucleic acid-based diagnostics, and in this context, thiazole orange has been the focus of intense study.

Thiazole Orange (TO)

Thiazole orange is a fluorescent asymmetric cyanine dye with an excitation peak at 514 nm and an emission peak at 533 nm. The molecule can be excited using a 488 nm laser and is composed of two heterocyclic ring systems (quinoline and benzothiazole) connected through a methine bridge (Figure 1). The fluorescence intensity of TO depends upon its conformation.[7] A planar state allows conjugation between the two aromatic systems- this is the fluorescent form; whereas rotation at the methine bridge produces a non-planar conformation, which is not fluorescent. In the presence of double stranded (ds) DNA, TO acts as an intercalator (or groove binder).[8,9] When intercalated, its fluorescent planar conformation is stabilised by stacking between base pairs.[10] This has led to the use of TO and its analogues such as SyBr Green and TOTO (Figure 1) in the fluorescence detection of dsDNA.[11] These molecules are essentially indiscriminate dsDNA binders.

In order to provide sequence-specific recognition of target nucleic acids, TO can be attached to

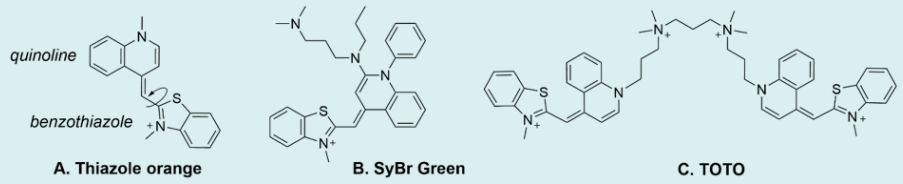

Figure 1: Structure of thiazole orange and other common analogues. The arrow indicates rotation around the methine bridge and only the planar state is fluorescent.

oligonucleotide probes. Such TO oligonucleotide conjugates have been used in combination with other fluorophores for highly sensitive, multi-colour detection of DNA and RNA targets.[12,13] The TO moiety has the useful additional property of strongly stabilising DNA duplexes, thus allowing shorter probes to

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be used and increasing discrimination between wild-type and mutant target sequences.[13] Pioneering work has been carried out by the Seitz group (FIT probes) using TO-labelled PNA[14] and TO-labelled oligonucleotides[15] for applications that include live cell studies.[16] The contributions to the field by Okamoto (ECHO probes)[17,18] and Wagenknecht (artificial TO DNA base)[19] are also ground-breaking. Furthermore, TO has been tethered to triplex forming oligonucleotides (TFOs) for sequence specific detection of DNA duplexes.[20] This approach provides remarkable triplex stabilisation and expands the potential range of duplex targets to include base pair inversions at biologically relevant pH. With these TO-TFOs, the stabilising effects of TO are additive, with the most stable triplex at neutral pH evidenced by a ΔT_m = +45 °C (compared to that of the unmodified TFO). This stability is accompanied by large enhancements in fluorescence (26-fold increase at pH 7) and quantum yields (up to 40-fold).[20]

The simplest way to incorporate TO into an oligonucleotide is to employ its NHS ester in post synthetic labelling of the corresponding amino-modified oligonucleotide. Various amino-modifiers are commercially available for incorporation into oligonucleotides at the termini or internally, and the labelling method is straightforward and generally high yielding. One example is the use of an internal amino-modifier C6 dT nucleobase (Figure 2A), which places the fluorescent label in the major groove upon duplex formation. Once the oligonucleotides are labelled, purification is carried out via HPLC (Figure 2B).

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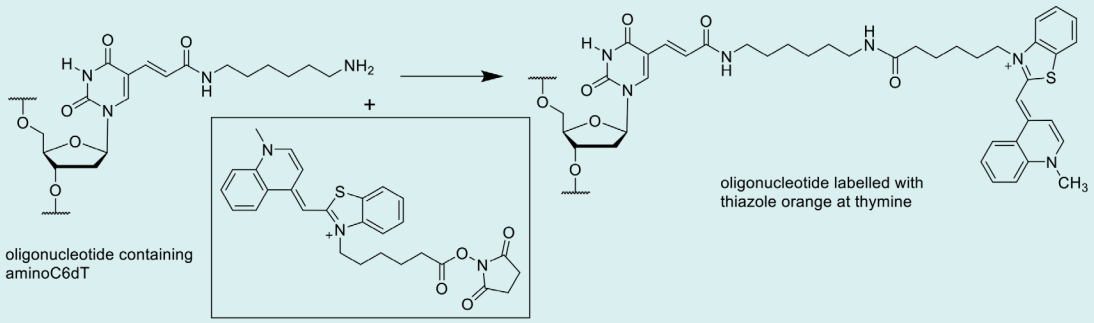
A.



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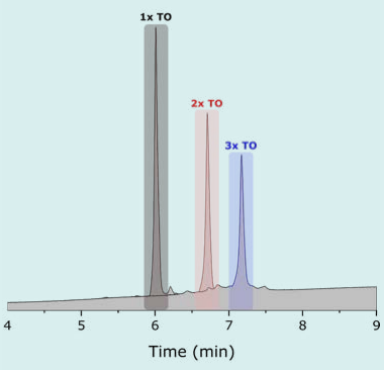

Figure 2. Post-synthetic labelling of oligonucleotides with TO. A. Labelling of internal thymine bases using TO NHS ester (in box) and amino-modifier C6 dT. B. UPLC analysis of purified oligonucleotides labelled with multiple TO units.[20] Retention time increases significantly as a function of the number of TO additions.

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New Product — Thiazole Orange NHS Ester

The Brown Group recently investigated the design of thiazole orange (TO) oligonucleotide probes for DNA and RNA.[1] The researchers evaluated a series of different sequences, TO conjugation locations, and TOs in the context of duplex stability, fluorescence, and CD. The data presented provide a wealth of information on TO probe design. Among the different TOs that were studied in this publication, a TO ligand that contained a hexanoic acid linker attached to the benzothiazole (Figure 1) was particularly interesting. This ligand was conjugated to short oligonucleotides containing a single internal amino-modifier. There were twelve DNA oligonucleotides in total, 4 different sequences multiplied by 3 different locations (2' position of ribose and 2 versions of 5-amino-modified-dU). Each one was hybridized to its complementary sequence for duplex stability and fluorescence analysis.

For these oligonucleotides, the results were significant and found to be dependent on the sequence, as well as where the TO was attached on the oligonucleotide. Melting temperatures were elevated by up to 14.6 °C, with an average of 10.1 °C relative to unmodified controls. This stabilization was relatively consistent regardless of whether the TO was on the nucleobase or the sugar. In addition, the hybridization was accompanied by a fluorescent enhancement of up to 8.6-fold, with an average of 3.9-fold. This "lighting up" effect on hybridization was even more significant when the probe was synthesized with a 2'-OMe backbone, mainly due to the fact that the 2'-OMe had much less single-stranded fluorescence. Unlike the melting temperature data, this enhancement notably increased when the TO was attached to the nucleobase. In order for researchers to better explore the use of TO in the fluorescence imaging of DNA and RNA, we are adding this particular TO NHS ester to our offerings.

In our hands, the TO NHS ester couples very well. In one test, TO was coupled to amino-modifier C6-T11. The oligonucleotide (previously desalted with a Glen Gel-Pak) was dissolved in aqueous sodium bicarbonate and mixed with a solution of TO NHS ester in DMSO (~5.5 eq). After incubation at room temperature for fifteen minutes, the reaction was desalted using another Glen Gel-Pak cartridge and analyzed by RP-HPLC (Figure 2). The amino-labeled oligonucleotide was completely consumed, giving a later eluting peak that contained TO. We also observed a second, much smaller (~4 %) TO-labeled oligonucleotide peak. ESI-MS analysis gave the desired TO-oligonucleotide mass as the main peak. In addition, the MS results suggested that the extra peak was a doubly labeled oligonucleotide. We found this second TO quite labile, an observation that would support 3'-OH esterification, and we were able to completely remove the extra TO with ammonium hydroxide at room temperature for an hour (Figure 2C).

References

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Item	Pack Size	Catalog No.
Thiazole Orange NHS Ester	5.4 mg	50-1970-23

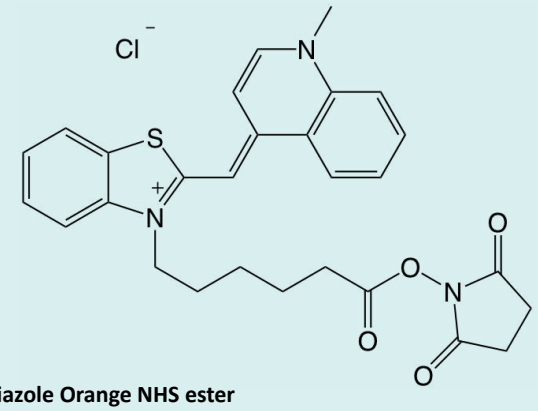

Figure 1. Thiazole Orange NHS ester

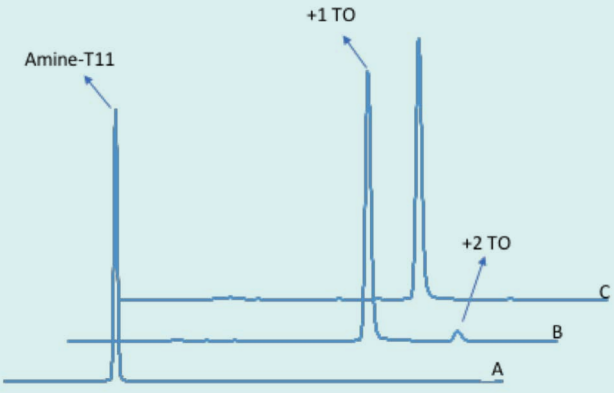

Figure 2. Labeling oligonucleotides with Thiazole Orange. RP-HPLC A254 results. TO NHS ester dissolved in DMSO was added to amino-modifier C6-labeled oligonucleotide in aqueous $NaHCO_3$ and allowed to react at room temperature. A) Amino-modifier C6-T11. B) Crude product. C) Crude product treated with ammonium hydroxide.

New Product — 2’-Fluoro-Inosine-CE Phosphoramidite

Inosine and 2’-deoxyinosine are non-standard, naturally occurring nucleotides that contain the nucleobase hypoxanthine. Inosine is found in tRNA wobble positions and processed mRNA, while 2’-deoxyinosine is a result of undesired 2’-deoxyadenosine deamination in DNA that needs to be repaired. Inosine is considered a universal base, as it can interact with each of the standard bases via two hydrogen bonds (Figure 1). The interactions in order of decreasing stability are I-C > I-A > I-T ≈ I-G.[1] The first three pairs are standard *anti-anti* interactions, whereas the last pair is an unusual I(*syn*) – G(*anti*) interaction.

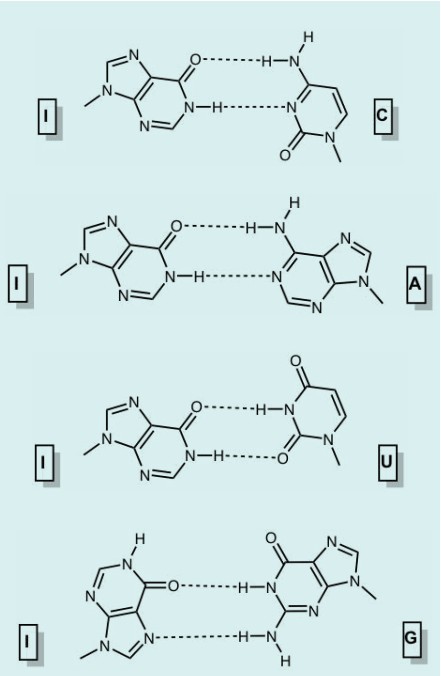

Figure 1. Base pairing interactions of inosine.

Due to these base pairing properties, inosine has been used in a number of applications predominantly involving either synthetic primers or probes.[2, 3] In cases where the target position is ambiguous, an inosine can be used to maximize hybridization as an alternative to degenerate oligonucleotides. This is particularly applicable when many ambiguous positions are present. A somewhat recent example of this is the use of inosine in 16S rRNA sequencing.[4]

16S rRNA sequencing is a standard method for the taxonomic identification of bacteria. The technique involves the use of PCR to generate an amplicon of a highly conserved region. The amplicon is then sequenced, and the resulting data is compared with public databases. Ben-Dov et al. used this technique to analyze the different types of bacteria present in an industrial waste water evaporation pond. The analysis was carried out with the common primer pair of 8F and 907R, as well as a variation of the same primer pair in which the 3’-terminal residues were replaced with 2’-deoxyinosine, 8F-I, and 907R-I, respectively. Sequencing data revealed that the diversity obtained from the 8F-I/907R-I pair was close to twice that of the 8F/907R pair. Interestingly, although the native primer pair gave less diversity, it did have results for one phylum of bacteria that was absent from the 8F-I/907R-I results, suggesting that the use of both primers with and without inosine may be the superior method in analyzing very diverse microbial samples.

To complement our existing DNA, RNA, and 2’-OMe versions of inosine, we are adding the 2’-F version (Figure 2). This phosphoramidite requires a coupling time of 3 min, and synthesized inosine-containing oligonucleotides can be deprotected with standard conditions. As is generally the case for 2’-F RNA, heating in AMA will lead to some degradation and should be avoided.

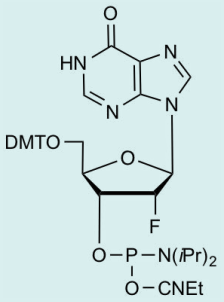

Figure 2. 2’-Fluoro-Inosine-CE Phosphoramidite

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Item	Pack Size	Catalog No.
2’-F-I-CE Phosphoramidite	100 µmol	10-3440-90
	0.25 g	10-3440-02

New Product — 2'-MOE RNA Phosphoramidites

Due to their sequence specific targeting nature, oligonucleotides have long been considered valuable tools in drug development. This is because once a therapeutic platform is fully developed, it can theoretically be applied to any target/disease by simply changing the oligonucleotide sequence. To date, platforms have included antisense and RNA interference, which have given rise to close to ten FDA-approved drugs.

Examining the sequences of these oligonucleotide drugs, reveals many different modifications to the sugar-phosphate backbone.[1, 2] These modifications 1) modulate the affinity of oligonucleotide drugs to their complementary targets, and/or 2) improve the stability/half-life of pharmaceutically active compound in cells and human fluids. Modified oligonucleotides are synthesized on the same synthesizers used for unmodified sequences and require special reagents, many of which are available at Glen Research. In addition to synthesis reagents for phosphorothioates (PS), 2'-OMe RNA (2'-OMe) and 2'-F RNA, Glen Research also offers other attractive modifications such as LNA, DNA PACE, 2'-OMe-RNA PACE, methyl phosphonates, 2'-FANA, DNA phosphorodithioates, 2'-OMe RNA phosphorodithioates and L-DNA, which can all be utilized in the development of oligonucleotide therapies to varying degrees.

A backbone modification that has been particularly prominent is 2'-O-methoxyethyl-RNA (2'-MOE).[3] Like other 2' modifications of ribose nucleotides (Figure 1), 2'-MOE favors the formation of A-form, RNA-like double helices, resulting in enhanced duplex stability when paired with RNA targets. In addition, 2'-MOE provides significant nuclease resistance and is relatively non-toxic. This combination has made 2'-MOE an attractive backbone for many oligonucleotide drug candidates, three of which have been approved by the FDA (Table 1 and Figure 2).[4, 5]

Kynamro treats homozygous familial hypercholesterolemia, a rare cholesterol disease, while Tegsedi treats nerve damage associated with hereditary transthyretin-mediated amyloidosis. Both drugs are 2'-MOE/DNA chimeras (gapmers) that contain ten central DNA nucleotides and five 2'-MOE nucleotides at each terminus. The two 2'-MOE regions enhance nuclease stability while ensuring that the oligonucleotide binds to its complementary sequence with appropriate affinity. The central DNA region, upon binding to target mRNA, forms a DNA/RNA heteroduplex, which is a substrate for RNase H cleavage. For Kynamro, mRNA cleavage results in a reduction of apolipoprotein B-100, a major component of low-density and very low-density lipoproteins. For Tegsedi, circulating concentrations of transthyretin are significantly reduced.

Spinraza was the first drug approved for spinal muscular atrophy, a rare disease that is the most common genetic cause of infant deaths. It is an eighteen nucleotide all 2'-MOE sequence, and unlike Kynamro and Tegsedi, Spinraza is a steric blocking oligonucleotide. When target mRNA is bound, no cleavage occurs. Instead, the binding of Spinraza onto its target mRNA interferes with splicing mechanisms, allowing an extra exon to be retained and rescuing the production of functional survival motor neuron 1 (SMN1) protein. When treated early with Spinraza, patients have much better outcomes, including superior motor coordination and reduced death rates.

In terms of balancing toxicity, affinity/off-target effects, and nuclease stability, the 2'-MOE backbone plays an important role in all three of the aforementioned drugs. As research with 2'- MOE continues to be conducted, Glen Research has decided to make the backbone more accessible by adding the 2'-MOE phosphoramidites of A, 5-Me-C, G and 5-Me-U (Figure 3).

Although 2'-MOE and 2'-OMe are very similar in terms of chemistry and functionality, 2'-OMe has been used in a much broader range of applications, some of which are unrelated to therapeutics. These include aptamers,[6] detection probes,[7] RNAi,[8] DNAzymes/ribozymes[9, 10] and CRISPR.[11] 2'-MOE should be applicable in all these contexts, as it has superior duplex stability and nuclease resistance. We hope that the addition of 2'-MOE will enable researchers to expand in their use of it.

The use of 2'-MOE reagents in oligonucleotide synthesis is relatively straightforward. A coupling time of 6 min is recommended, and oligonucleotides that contain these residues can be deprotected following our standard procedures. It is important to note that methylamine should not be used with 2'-MOE-Bz-5-Me-C, in order to avoid methylation of the N4.

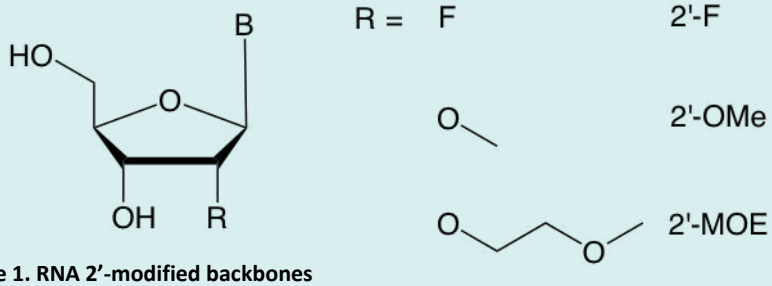

Figure 1. RNA 2'-modified backbones

Drug	Condition	Mechanism	FDA Approval
Kynamro (Mipomirsen)	Familial hypercholestolemia	RNase H	2013
Spinraza (Nusinersen)	Spinal muscular atrophy	Splicing modulation	2016
Tegsedi (Inotersen)	Hereditary transthyretin-mediated amyloidosis	RNase H	2018

Table 1. 2'-MOE oligonucleotide drugs approved by the FDA

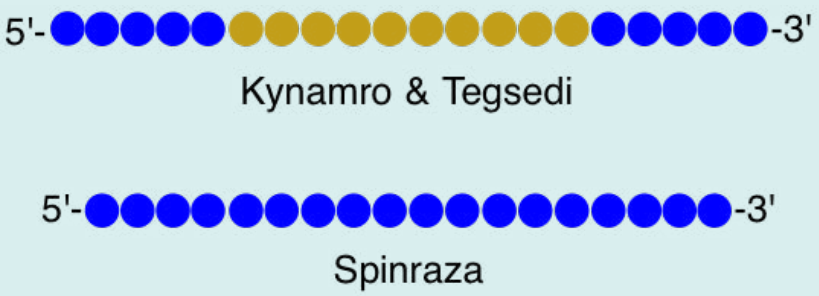

Figure 2. Structure schemes of three 2'-MOE drugs. Blue denotes 2'-MOE; tan denotes DNA; all phosphates are PS.

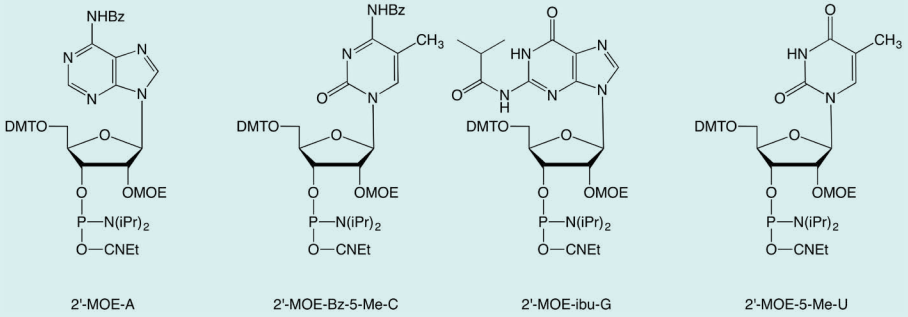

Figure 3. 2'-MOE Phosphoramidites

Item	Pack Size	Catalog No.
2'-MOE-A-CE Phosphoramidite	0.5 g	10-3200-05
	1.0 g	10-3200-10
	2.0 g	10-3200-20
2'-MOE-Bz-5-Me-C-CE Phosphoramidite	0.5 g	10-3211-05
	1.0 g	10-3211-10
	2.0 g	10-3211-20
2'-MOE-ibu-G-CE Phosphoramidite	0.5 g	10-3220-05
	1.0 g	10-3220-10
	2.0 g	10-3220-20
2'-MOE-5-Me-U-CE Phosphoramidite	0.5 g	10-3231-05
	1.0 g	10-3231-10
	2.0 g	10-3231-20

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Application Note — Trimer Phosphoramidites

Glen Research offers a unique line of products known as trimer phosphoramidites. These are trinucleotide reagents that allow customers to effectively synthesize oligonucleotides based on amino acid codons rather than individual nucleotides.[1] For researchers looking to generate oligonucleotide libraries for mutagenesis, these reagents avoid stop codons and amino acid redundancy. In theory, such libraries can be obtained via a split and pool synthesis workflow without trimer phosphoramidites, but this is very awkward to perform in practice for a large number of codons or longer codon-containing regions.

Glen Research offers a total of 29 trimer phosphoramidites that cover all 20 standard amino acid codons in the sense and antisense directions and are optimized for *E. coli* expression. We also offer two standard trimer phosphoramidite mixes, both for sense trimers: Mix 2, 13-1992-xx, which contains an equal mixture of 19 codons (no cys), and Mix 1, 13-1991-xx, which contains an equal mixture of 20 codons. For other ratios, we can custom prepare ready-to-use reagents as desired. Alternatively, customers may purchase the trimers individually and then construct their own mixes. Those who do decide to pursue this latter option will need to be mindful of the different coupling rates that the various trimers exhibit, as detailed in Glen Report articles 16.25 and 25.12.

Over the years, our customers have published some exciting results using libraries synthesized with our trimer phosphoramidite mixes. The Regan Group at Yale developed a new screening method for protein affinity agents.[2] The technique is based on splitting green fluorescent protein (GFP) into 2 halves and fusing a peptide library and a target peptide to the GFP fragments (Figure 1). During screening, if a member of a library binds to the desired peptide, then the two halves of GFP are brought together resulting in detectable fluorescence. The researchers constructed a peptide library based on the tetratricopeptide repeat (TPR) as a framework (34 amino acids, aa). In the library, seven positions were randomized with our Trimer Phosphoramidite Mix 2 (no cys). This library was fused with the C-terminal fragment of GFP (CGFP) and screened against several targets in *E. coli*: c-Myc epitope tag (10 aa), full length Dss1 (70 aa), and the C-terminal epitope of Dss1 (19 aa), each of which was fused to the N-terminal fragment of GFP (NGFP). Screening was performed in *E. coli*, and bacteria that were fluorescent were identified and isolated by fluorescence activated cell sorting (FACS). For each of the targets, two rounds of iterative selection successfully isolated binders that bound specifically with K_ds in the micromolar range.

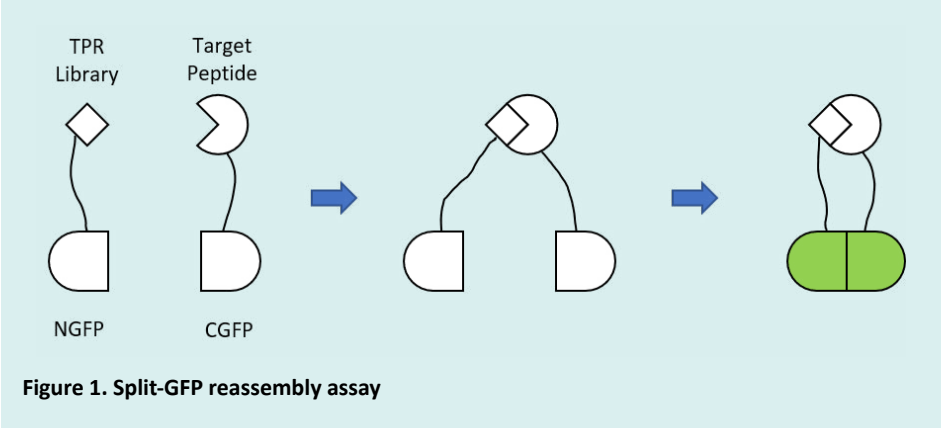

Figure 1. Split-GFP reassembly assay

In another investigation, the Sidhu Group at the University of Toronto examined the importance of certain regions of antibodies in terms of antigen recognition.[3] Antibodies are typically "Y-shaped" and consist of 2 heavy and 2 light chains held together by disulfide bonds (Figure 2). At the top of the Y are 2 identical antigen binding domains, and each of these domains contains 6 complementarity-determining regions (CDRs), 3 from the light chain and 3 from the heavy chain. In nature, the third CDR of the heavy chain (CDR-H3) has been shown to be the most important, although it is unclear why. Notably, the location of the third CDR of the light chain (CDR-L3) is positioned to potentially play as big of a role as CDR-H3, meaning that genetics may be the underlying reason for the importance of CDR-H3. The researchers assembled antigen-binding libraries that contained diversity generated with a Custom Trimer Phosphoramidite Mix of Tyr/Ser/Gly/Ala/Phe/Trp/His/Val/Pro 5:4:4:2:1:1:1:1:1. Using this library and phage display, the researchers were able to generate many functional antibodies for a range of different antigens. These synthetic antibodies were analyzed in terms of antigen binding and sequence content, and the results were the opposite of what is observed in nature. CDR-L3 was shown to be more important than CDR-H3. To follow up on these experiments, shotgun alanine-scanning and X-ray crystallography were used to further characterize the CDR to antigen interactions.

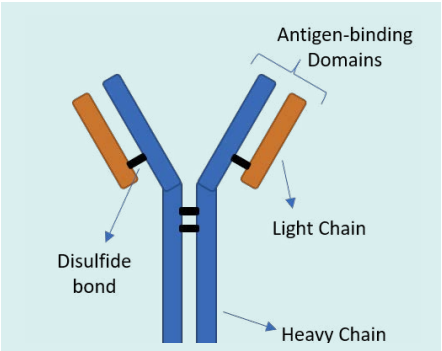

Figure 2. Antibody structure

In a third publication, the Ellington Group at the University of Texas at Austin

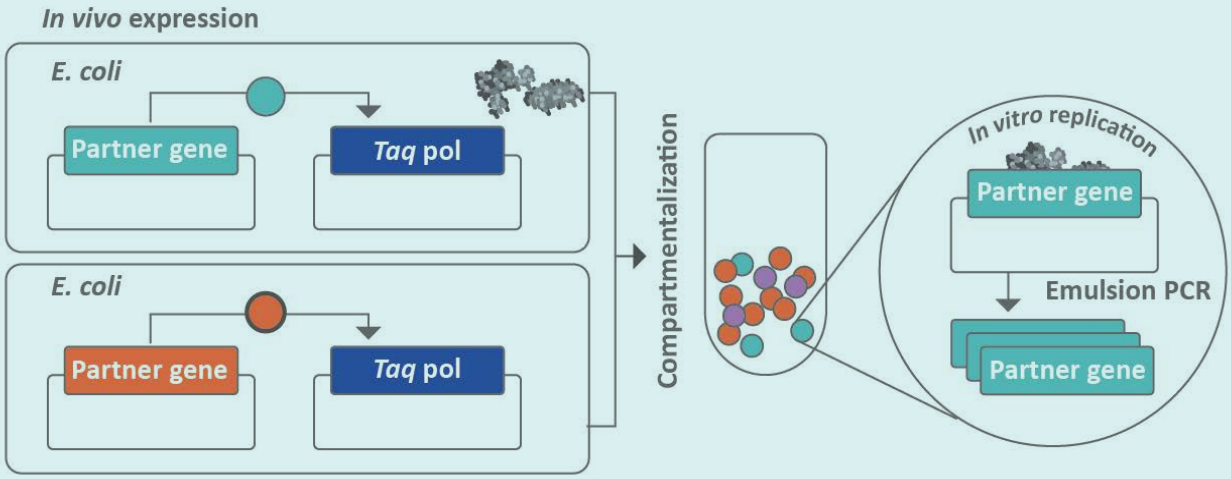

Figure 3. General compartmentalized paired replication concept. Partner genes are shown as either active (green) or inactive (red).

described a directed evolution method called compartmentalized partnered replication.[4] This technique couples the expression of a protein of interest to the expression of Taq DNA polymerase (Figure 3). The authors chose T7 RNA polymerase (T7 RNAP) as a proof of principle. In this setup, Taq DNA polymerase expression would be dependent on T7 RNAP binding onto the promotor. The investigation began with the generation of a plasmid library of T7 RNA polymerase variants, where six of the amino acids in the specificity loop were randomized with oligonucleotides constructed from Trimer Phosphoramidite Mix 1. This library, along with Taq DNA polymerase plasmids, was transformed into *E. coli*. The bacteria were then transferred to a water-in-oil emulsion in which a large number of water droplets or "compartments" were present. Each droplet statistically contained a single cell as well as PCR primers. Only the cells with desirable mutants would drive expression of Taq DNA polymerase. To measure the amount of Taq DNA polymerase produced, PCR was carried out on the emulsions. Heating during PCR lysed the cells, and the primers that were in the droplet directed the amplification of the T7 RNAP. Each droplet was effectively a single screening experiment. After PCR, the emulsion was collapsed, and the PCR products were isolated. The resulting mutant pool was then used to construct an enriched plasmid pool for another round of compartmentalized partnered replication. After 4 rounds of this, the sequence pool was very similar to the native T7 RNAP sequence.

As a follow up experiment, the aforementioned original mutant library was paired with a mutated promotor for Taq DNA polymerase. This required 16 more rounds of selection, several of which involved the use of error-prone PCR to derive mutants that were not available in the original library. In the end, a mutant T7 RNAP/mutant Taq DNA polymerase promotor pair that gave comparable expression levels to the respective native/native pair was characterized.

In each of these investigations, the use of trimer phosphoramidite mixes allowed researchers to create defined sequence libraries that maximized the efficiency of the screening methods they used. No sequence space was lost due to undesired amino acids or stop codons, and the ratios of the amino acids were fully customizable. For those who are still using degenerate oligonucleotides for mutagenesis library construction, it might be a good time to consider using trimer phosphoramidites.

References

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2. M.E. Jackrel, A.L. Cortajarena, T.Y. Liu, and L. Regan, ACS Chem Biol, 2010, **5**, 553-62.
3. H. Persson, et al., J Mol Biol, 2013, **425**, 803-11.
4. J.W. Ellefson, et al., Nat Biotechnol, 2014, **32**, 97-101.

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Item	Pack Size	Catalog No.
Trimer Phosphoramidite Mix 1	50 µmol	13-1991-95
	100 µmol	13-1991-90
Trimer Phosphoramidite Mix 2	50 µmol	13-1992-95
	100 µmol	13-1992-90
Custom Trimer Phosphoramidite Mix	Custom	13-9999-SP
AAA Trimer Phosphoramidite	50 µmol	13-1000-95
	100 µmol	13-1000-90
AAC Trimer Phosphoramidite	50 µmol	13-1001-95
	100 µmol	13-1001-90
ACC Trimer Phosphoramidite	50 µmol	13-1011-95
	100 µmol	13-1011-90
ACT Trimer Phosphoramidite	50 µmol	13-1013-95
	100 µmol	13-1013-90
AGA Trimer Phosphoramidite	50 µmol	13-1020-95
	100 µmol	13-1020-90
ATC Trimer Phosphoramidite	50 µmol	13-1031-95
	100 µmol	13-1031-90
ATG Trimer Phosphoramidite	50 µmol	13-1032-95
	100 µmol	13-1032-90
CAG Trimer Phosphoramidite	50 µmol	13-1102-95
	100 µmol	13-1102-90
CAT Trimer Phosphoramidite	50 µmol	13-1103-95
	100 µmol	13-1103-90
CCA Trimer Phosphoramidite	50 µmol	13-1110-95
	100 µmol	13-1110-90
CCG Trimer Phosphoramidite	50 µmol	13-1112-95
	100 µmol	13-1112-90
CGG Trimer Phosphoramidite	50 µmol	13-1122-95
	100 µmol	13-1122-90
CGT Trimer Phosphoramidite	50 µmol	13-1123-95
	100 µmol	13-1123-90
CTG Trimer Phosphoramidite	50 µmol	13-1132-95
	100 µmol	13-1132-90
GAA Trimer Phosphoramidite	50 µmol	13-1200-95
	100 µmol	13-1200-90
GAC Trimer Phosphoramidite	50 µmol	13-1201-95
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GAT Trimer Phosphoramidite	50 µmol	13-1203-95
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GCA Trimer Phosphoramidite	50 µmol	13-1210-95
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GCG Trimer Phosphoramidite	50 µmol	13-1212-95
	100 µmol	13-1212-90

Item	Pack Size	Catalog No.
GCT Trimer Phosphoramidite	50 µmol	13-1213-95
	100 µmol	13-1213-90
GGT Trimer Phosphoramidite	50 µmol	13-1223-95
	100 µmol	13-1223-90
GTA Trimer Phosphoramidite	50 µmol	13-1230-95
	100 µmol	13-1230-90
GTT Trimer Phosphoramidite	50 µmol	13-1233-95
	100 µmol	13-1233-90
TAC Trimer Phosphoramidite	50 µmol	13-1301-95
	100 µmol	13-1301-90
TCT Trimer Phosphoramidite	50 µmol	13-1313-95
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TGC Trimer Phosphoramidite	50 µmol	13-1321-95
	100 µmol	13-1321-90
TGG Trimer Phosphoramidite	50 µmol	13-1322-95
	100 µmol	13-1322-90
TTC Trimer Phosphoramidite	50 µmol	13-1331-95
	100 µmol	13-1331-90
TTT Trimer Phosphoramidite	50 µmol	13-1333-95
	100 µmol	13-1333-90

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Technical Snippets

How much DBCO is lost if standard iodine oxidation is used?

The answer is it varies. Iodine will cause DBCO to be cleaved from the oligonucleotide, and the rate will be dependent on temperature, iodine concentration and exposure time. For internally located DBCO groups, numerous cycles of iodine exposure can be very problematic. However, even if the DBCO is only added as a 5' label, the amount of DBCO loss can still be undesirable depending on how the last oxidation step is carried out. To completely avoid this side reaction, we recommend the use of CSO oxidizer for the oxidation of all DBCO-containing oligonucleotides as discussed earlier (https://www.glenresearch.com/reports/gr27-17). Alternatively, we offer DBCO-sulfo-NHS Ester that completely bypasses the exposure of DBCO to oxidization reagents.

Products: 5'-DBCO-TEG Phosphoramidite, 10-1941
DBCO-dT-CE Phosphoramidite, 10-1539
DBCO-Serinol Phosphoramidite, 10-1998

Why is the RP-HPLC chromatogram for phosphoramidites often two peaks and sometimes even more?

This is due to the presence of diastereomers. When phosphorus has a lone pair of electrons and three different groups attached to it, as is the case for a phosphoramidite, it is a stereocenter. For a modifier phosphoramidite without other stereocenters, one would typically see only one peak (Figure 1). For a standard DNA phosphoramidite, however, D-2'-deoxyribose causes the phosphoramidite peak to split into two. For an extreme example, our trimer phosphoramidites have as many as eight peaks due to the presence of three phosphorus stereocenters. With all these stereocenters being used to construct oligonucleotides, readers may wonder whether oligonucleotides are a large mix of stereoisomers. Fortunately, that is not the case. After oxidation and deprotection, the phosphorus in the phosphodiesters are no longer stereocenters.

Products: All phosphoramidites

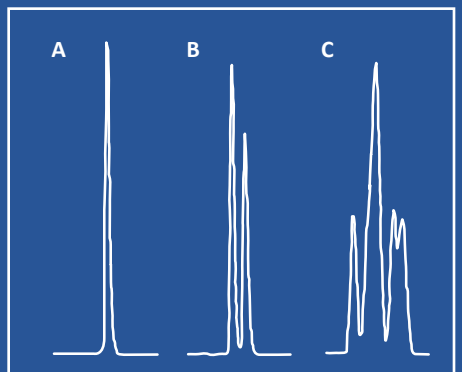

Figure 1. RP-HPLC of three different phosphoramidites. A. 5'-amino-modifier C6; B. dT-CE phosphoramidite; C. TCT trimer phosphoramidite. The relative scale of the x-axis is identical for all three chromatograms.

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